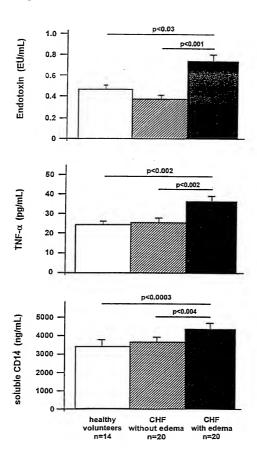
PCT/EP00/02062

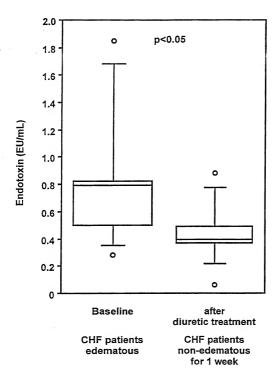
100

Figure 1:



. .





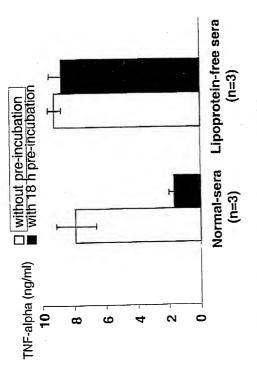


Figure 3: Lipoprotein-free serum lacks LPS-neutralizing activity

Sera were incubated with 3 ng/ml LPS and added to human monocytes directly or after a 18 h pre-incubation time

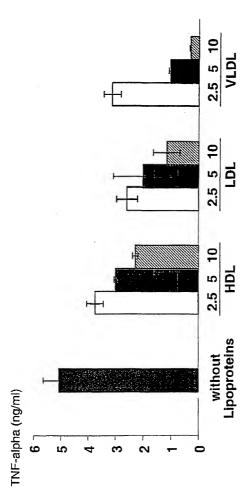
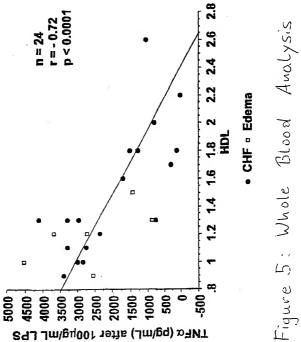


Figure 4: Lipoproteins including HDL, LDL and VLDL inhibit LPS-induced TNF-release of monocytes. The effects of LDL and VLDL are even stronger than that of HDL. In all xperiments: n=3

Lipoproteins were added to Lipoprotein-free Serum (5 %) and incubated for 17 h with 3 ng/ml LPS before addition to monocytes





TNF-alpha (ng/ml)



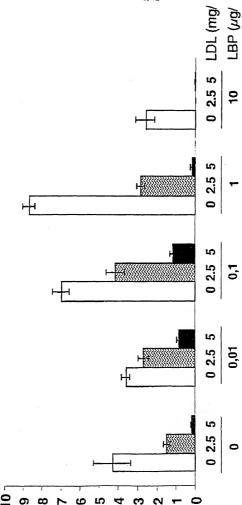


Figure 6: In the presence of elevated LBP-concentrations lipoproteins show emhanced LPS-LBP and LDL were pre-incubated for 17 h before cell stimulation with 3 ng/ml LPS neutralization capacity. In all experiments: n=3.

TNF-alpha (ng/ml)

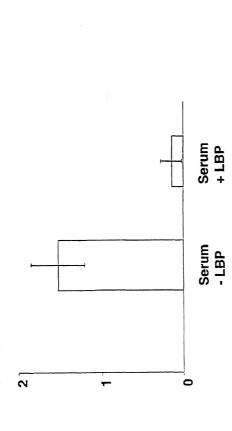


Figure 7: Addition of LBP to lipoprotein containing serum reduces LPS-mediated TNF production (n=3).

Normal serum +/- 10 µg/ml LBP was incubated with 3 ng/ml LPS for 16 h, and then added to monocytes



Figure 8: LPS - Neutralisation by UDCA in whole blood of 4 healthy subjects

|   | Con  | Control D | Control J  | rol J     | Control Ch | ol Ch     | Control F | rol F     |
|---|------|-----------|------------|-----------|------------|-----------|-----------|-----------|
| N. C.             | TNF  | 1L6       | TΝFα       | IL6       | TΝFα       | 1L6       | TNFα      | 1L6       |
| Measurements by infinitie                             | J.d  | pg/ml     | lm/gd      | ш         | lm/gd      | E I       | lm/gd     | ш         |
| Control, blood alone (Con)                            | 6.7  | \$ 5      | 4.6        | \$ \$     | 45,        | ∞ ç       | 15.4      | Α ξ       |
| Con + 50 <i>pg/mt</i> LPS<br>Con + BPI (1μg/ml)       | 4, 4 | 301<br>\$ | 456<br>6.9 | 380<br>\$ | 6.9        | 300<br>\$ | 8         | 48/<br>\$ |
| Blood with UDCA 1 me/ml (1% ethanol)                  | 4.8  | \$        | 9          | 6.5       | 4          | 8.5       | 4>        | \$        |
| + LPS + UDCA  | 4    | 5.6       | 2          | ζ,        | 6.4        | 9.1       | <b>^</b>  | Δ.        |
| + LPS + Ethanol 1% (no UDCA)                          | 126  | 119       | 315        | 286       | 407        | 318       | 430       | 408       |
| 17 - 70 W 1-7 - 801 Y Dall 11; 1                      | 10.6 | ζ.        | 14         | \$        | 42.7       | 463       | 16        | \         |
| Blood With ODCA 100/pg/mi (0.178 emano)<br>+1.PS+UDCA | 114  | 114       | 41.3       | 20.4      | 49.6       | 306       | 397       | 419       |
| + LPS + Ethanol 0.1% (no UDCA)                        | 265  | 230       | 221        | 263       | 599        | 375       | 569       | 414       |
| Blood with UDCA 10 µg/ml (0.01%                       | 8.5  | \$        | 8.3        | \$        | 13.7       | 6         | 12.3      | \$        |
| ethanot)<br>+ LPS                                     | 279  | 248       | 432        | 358       | 617        | 400       | 009       | 499       |

Figure 9: LPS - Neutralisation by UDCA in whole blood in patient 1

|   | P1 /                | P1/-1              | P1/0                | 0/                 | P1/1  | /1   | P1/2 | 12    | P1   | P1/5  |
|---|---------------------|--------------------|---------------------|--------------------|-------|------|------|-------|------|-------|
| Moonwoonen hay Immulife   | TNF                 | IL6                | TNFα IL6            | 9TI                | TΝFα  | IL6  | TΝFα | 1L6   | TNFα | 1L6   |
| Measurements by minimum   | lm/gd               | ml                 | pg/                 | pg/ml              | lm/gd | lm   | pg   | pg/ml | pg   | pg/ml |
| Control, blood alone (Con)<br>Con + 50 pg/ml LPS<br>Con + BPI ([µg/ml)                    | 28.7<br>878<br>29.7 | 11.2<br>573<br>< 5 | 70.2<br>938<br>15.1 | 35.2<br>723<br>< 5 |       |      |      |       |      |       |
| Blood with UDCA I mg/ml (1% ethanol)<br>+ LPS + UDCA<br>+ LPS + Ethanol 1% (no UDCA)      | 4 × ×               | 12.8               | 10.<br>6.4<br>648   | 9.8<br>6.0<br>278  |       |      |      | 14    |      |       |
| Blood with UDCA 100µg/ml (0.1% ethanol)<br>+ LPS + UDCA<br>+ LPS + Ethanol 0.1% (no UDCA) | 8.5                 | 7.8                | 24.5<br>692<br>886  | 19.7<br>773<br>580 |       |      |      |       |      |       |
| Blood with UDCA 10 µg/ml (0.01% ethanol)<br>+ L.P.S                                       | 38.0<br>952         | 11.4               | 93.0<br>1013        | 45.7<br>853        |       |      |      |       |      |       |
| Plasma levels   |                     |                    | 9.1                 | 26.7               | 8.3   | 28.8 | <4   | 20.5  | 5.8  | 28.1  |

Figure 10: LPS - Neutralisation by UDCA in whole blood in patient 2

|  | P2/-1      | /-1   | P2          | P2 / 0 |
|--|------------|-------|-------------|--------|
| Messurements by Immilite                 | ΤΝΓα       | 1L6   | $TNF\alpha$ | IL6    |
| Measurements by minimize                 | /Bd        | pg/ml | /Bd         | pg/ml  |
| Control, blood alone (Con)               | 29.0       | 10.5  | 47.5        | 25.6   |
| Con + 50 pg/ml LPS                       | 731        | 544   | 807         | 287    |
| Con + BPI (1µg/ml)                       | 17.0       | \$    | 8.6         | < 5    |
| Blood with UDCA 1 mg/ml (1% ethanol)     | <b>4</b> > | 10.1  | <b>*</b> >  | 7.8    |
| + LPS + UDCA                             | 6.7        | < 5   | 4 >         | 5.4    |
| + LPS + Ethanol 1% (no UDCA)             | 569        | 419   | 540         | 405    |
|  | 146        | 7.0   | 757         | 10.5   |
| Blood with UDCA 100µg/mi (0.17% eindhoi) | 27.1       | 2,72  | 150         | 301    |
| + LPS + Ethanol 0.1% (no UDCA)           | 712        | 546   | 993         | 788    |
| Blood with UDCA 10 µg/ml (0.01%          | 42.4       | 26.2  | 54.1        | 32.0   |
| ethanol)<br>+ LPS                        | 712        | 622   | 744         | 532    |
| Plasma levels                            | 4.9        | 9.9   | <4          | 9.7    |

Figure 11: LPS - Neutralisation by UDCA in whole blood in patient 3

|  | P3 /-1 | -1   | P3         | P3 / 0 |  |
|--|--------|------|------------|--------|--|
| Moonimonts by Immilite                   | TNFα   | 1L6  | TΝFα       | IL6    |  |
| Measurements by minimine                 | pg/ml  | ml   | þ          | pg/ml  |  |
| Control. blood alone (Con)               | 43.1   | 7.9  | 52.1       | 12.9   |  |
| Con + 50 pg/ml LPS                       | 450    | 378  | 490        | 346    |  |
| Con + BPI (1μg/ml)                       | 16.4   | < 5  | 10.0       | < 5    |  |
|  |        |      |            |        |  |
| Blood with UDCA 1 mg/ml (1% ethanol)     | 6.5    | 10.4 | 4          | 9.1    |  |
| +LPS+UDCA                                | 4 >    | 10.3 | <b>4</b> × | 10.7   |  |
| + LPS + Ethanol 1% (no UDCA)             | 208    | 108  | 288        | 169    |  |
|  |        |      |            |        |  |
| 1  | 12     | 70   | 21.7       | 7 8    |  |
| Blood With UDCA Joshg/mi (0.1% ethanoi)  | 1.71   | 200  | 241        | 203    |  |
| + LF3 + UDCA                             | 70.0   | 5.50 | 147        | 797    |  |
| + Lr3 + Ethanol 0.1% (no ODCA)           | 383    | C97  | 4448       | 340    |  |
|  | 111    | 0    | 7.00       | 101    |  |
| Blood with UDCA 10 µg/ml (0.01% ethanol) | 34.7   | 8.0  | 39.4       | 10.7   |  |
| +LPS                                     | 375    | 310  | 468        | 300    |  |
|  |        |      |            |        |  |
| Plasma level                             | 13.0   | 17.1 | 10.2       | 15.9   |  |
|  |        |      |            |        |  |



Figure 12: LPS - Neutralisation by UDCA in whole blood in patient 4

|  | P4/-1      | /-1    | P4/0         | 0/   | P4         | P4 / 1 | P4          | P4/2  |
|--|------------|--------|--------------|------|------------|--------|-------------|-------|
| 7 ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) ( ) (    | TΝFα       | IL6    | $TNF\alpha$  | 9TI  | TΝFα       | 9TI    | $TNF\alpha$ | IL6   |
| Measurements by infinitite                 | lm/gd      | m<br>I | pg/ml        | III  | bg         | pg/ml  | pg          | pg/ml |
| Control. blood alone (Con)                 | 34.5       | 10.4   | 29.5         | 10.2 | 4.7        | < 5    | 4.0         | < 5   |
| Con + 50 pg/ml LPS                         | 224        | 98.4   | 172.0        | 77.5 | 156        | 88.9   | 100         | 46.8  |
| Con + BPI (1µg/ml)                         | 18.1       | <5     | 33.5         | 7.1  | <b>4</b> > | < 5    | 4           | < 5   |
| Blood with UDCA 1 mg/ml (1% ethanal)       | < 4        | 8.2    | < <b>4</b> > | 8.2  | 4 >        | < 5    | ^<br>4      | 8.8   |
| + LPS + UDCA                               | <b>4</b> × | 9.9    | <b>4</b> >   | 9.5  | < <b>4</b> | 9.9    | <b>4</b> >  | 6.3   |
| + LPS + Ethanol 1% (no UDCA)               | 132        | 48.0   | 98.5         | 68.4 | 6.99       | 35.6   | 49.5        | 26.5  |
| Discolarith IIDCA 100116/ml (0.19% orbano) | 83.7       | 49.6   | 44.5         | 0.09 | 4.2        | < 5    | 4 >         | < 5   |
| + LPS + UDCA                               | 242        | 257    | 55.8         | 61.0 | 89.7       | 36.6   | 39.9        | 34.6  |
| + LPS + Ethanol 0.1% (no UDCA)             | 154        | 130    | 136.0        | 159  | 141        | 82.7   | 120         | 81.0  |
| Blood with UDCA 10 ug/ml (0.01% ethanol)   | 42.3       | 13.6   | 174          | 72.0 | 10.9       | <5     | 5.2         | < > 5 |
| +LPS                                       | 188        | 80.2   | 278          | 166  | 163        | 88.7   | 92.6        | 53.4  |
| Plasma levels                              | 8.1        | <5     | 4>           | < 5  | 4          | < 5    | 4.7         | < 5   |
|  |            |        |              |      |            |        |             |       |

SUBSTITUTE SHEET (RULE 26)